Formamide can dramatically improve the specificity of PCR

Gobinda Sarkar, Stephen Kapelner and Steve S.Sommer*
Department of Biochemistry and Molecular Biology, Mayo Clinic/Foundation, Rochester, MN 55905, USA

Submitted September 24, 1990

The polymerase chain reaction (1) has become an indispenable tool of molecular biology. PCR of genomic segments from mammals typically generates acceptable specificity. However, specificity is much more difficult to achieve for segments of high GC content. In one instance for which the GC content was 74%, specificity was achieved with 7-deaza-2′-deoxyguanosine 5′-triphosphate (dc^{7}GTP) and a higher annealing temperature (2). Two rounds of PCR with nested primers also can be effective (3), but additional primers must be synthesized.

We hav been examining five GC-rich segments (ca. 55%) of the human dopamine D2 receptor gene (4). Despite the use of PCR primers with about 50% A+T, poor specificity was observed. Two of the segments are shown in Figure 1. Replacement of 75% of dGTP by dc^{7}GTP (as recommended in ref. 2) with or without an increase in annealing temperature reduced the intensity of ethidium bromide staining without significantly improving specificity (lanes D7). The denaturant DMSO did not give the desired specificity (lanes D1−D4). However, inclusion of formamide (lanes F1−F4) in the PCR eliminated most of the nonspecific products and increased the efficiency of the amplification. In four of the five GC-rich segments formamide dramatically improved specificity while dc^{7}GTP and DMSO were ineffective or partially effective. Direct sequencing by the genomic amplification with transcript sequencing (5) verified that the desired products had been amplified. Formamide was also effective in regions of p53, another GC rich gene in which specificity of amplification was a problem.

In conclusion, formamide was found to be a simple and inexpensive method for increasing the specificity of PCR.

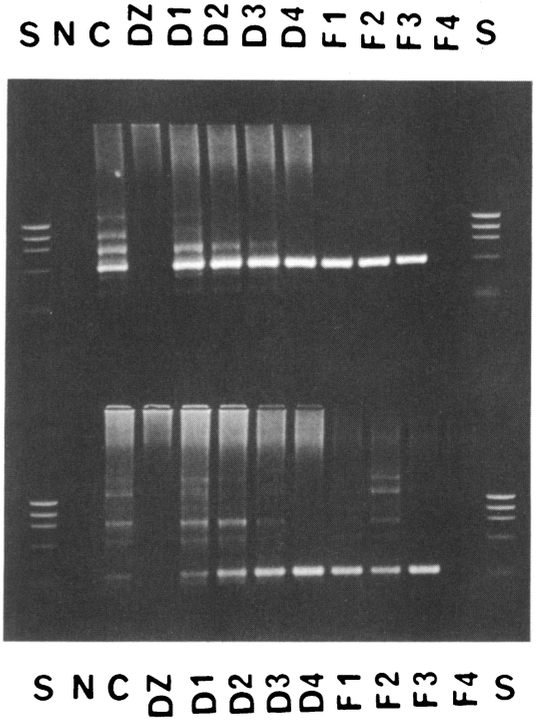

FIGURE 1. Specificity of amplification of segments of the human D2 receptor gene in response to various additions. PCR was performed under standard conditions (6). In brief, 30 cycles of PCR (in 20 μl volume) were performed with 1 min at 94°C, 2 min at 50°C and 3 min at 72°C with or without the denaturants. 5μl of each sample was loaded onto a 1.5% agarose gel. In the upper panel, a segment of 579 bp is expected and for the lower panel a segment of 303 bp is expected. Lanes S − size standards obtained by digesting ΦX174 DNA with *Hae*III; N − no DNA; C − control PCR under standard conditions; DZ − PCR in presence of dc^{7}GTP; D1, D2, D3 and D4, PCR in presence of 2.5%, 5%, 10% and 15% DMSO, respectively; F1, F2, F3 and F4, PCR in presence of 1.25%, 2.5%, 5% and 10% formamide, respectively.

REFERENCES

1. Saiki,R.K. *et al.* (1988) *Science* **239**, 487−494.
2. McConlogue,L. *et al.* (1988) *Nucl. Acids Res.* **16**, 9869.
3. Haqqi,T.M. *et al.* (1988) *Nucl. Acids Res.* **16**, 11844.
4. Grandy,D.K. *et al.* (1989) *Proc. Natl. Acad. Sci. USA* **86**, 9762−9766.
5. Sommer,S.S. *et al.* (1990) In *PCR Protocols: A Guide to Methods and Applications*. Academic Press, pp. 197−205.
6. Sarkar,G. and Sommer,S.S. (1989) *Science* **244**, 331−334.
7. Hung,T. *et al.* (1990) *Nucl. Acids Res.* **18**, 4953.

Note in Proof: Tetramethylammonium chloride has recently been reported to enhance the specificity of PCR (7).

* To whom correspondence should be addressed